triglyceride fraction of the several areas of the aorta and serum were similar. It has been observed that there is an increase in the triglyceride and phospholipid content of the intima as atherosclerosis develops, as well as in free and ester cholesterol(10). This, plus the similarity of the triglyceride fatty acid composition in media, serum and plaques suggests that there is an indiscriminate deposition of triglyceride in the aorta. While there were some differences in fatty acid distribution of the phospholipid fraction in the several areas of the aorta and serum, it should be pointed out that that fraction was heterogeneous since it contained lecithins, sphingomyelins and cephalins. The differences in the percentages of the polyunsaturated fatty acids in that fraction of the several tissues studied might be due to the greater abundance of a specific phospholipid. The results of the present study stress the need for further study of the origin and metabolism of the cholesterol ester fraction in the aorta.

Summary. The cholesterol ester, triglyceride and phospholipid fatty acid composition (gas-liquid chromatography) of aortic media, thickened intima and plaque material were determined in 6 human subjects. For comparison, the fatty acid composition of those fractions in serum was determined in 6 subjects (aged 55-70 years) with occlusive atherosclerosis in a good nutritional status. The triglyceride fraction of those tissues and serum were similar in their fatty acid composition. Some slight differences were noted in the fatty acid composition of the phospholipid fractions, most notably in the arachidonic and long chain fatty acids. The cholesterol ester fraction of the tissues studied showed the greatest differences in fatty acid composition. Both early and advanced plaques had significantly less linoleic and more oleic acid in that fraction than serum or media. Media and serum CEFA were similar in their fatty acid composition. The significance of these findings in relation to atherogenesis is discussed.

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Evaluation of Respiratory Mutants for Selecting Compounds for Cancer Chemotherapy Study.*† (26210)

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Many investigators, in their search for systems which might respond similarly to tumor tissue because of some parallelism of metabolism, have attempted to establish a preliminary screen for cancer chemotherapy utilizing organisms which might be induced to preferentially use fermentative metabolism. Studies with anaerobes by Bradner and Clarke(1) as well as by DiPaolo and Rosenfield(2) indicated that results with these sys-

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tems are not actually superior to the results obtained with aerobes.

Respiratory mutants of several yeast strains obtained by incorporating acriflavine (2) into the growth medium differ from the wild type visibly and in their ability to utilize glucose by the oxidative process. It has been theorized that a respiratory mutant which has a change in the mode of utilization of glucose might be an excellent tool for selecting cancer chemotherapeutic agents. Gause(3,4) has reported that mutants with impaired oxidation are selectively inhibited by anticancer preparations and therefore are of value for evaluating new anticancer compounds. Unfortunately, most of the compounds tested by us(2,5) failed to inhibit preferentially the respiratory yeast mutants obtained from several strains of yeast.

More recently Gause has made available through the American Type Culture Collection his *Staphylococcus aureus* and 2 "mutants" derived therefrom (ATCC #13679 to 13681). This paper reports results obtained with a selected group of drugs using our 2 yeast strains and the *S. aureus* strains from ATCC.

Materials and methods. A series of 16 compounds reported as inhibitory on experimental neoplasms and of interest to clinical cancer chemotherapy, was tested by an agar-disc assay method in Petri dishes for their preferential growth inhibition of yeast respiratory mutants and *S. aureus* (ATCC #13680, 13681).

In the yeast study, results obtained with respiratory mutants and the wild type were compared. Mutant colonies were picked at random after being grown on agar medium supplemented with acriflavine. For sensitivity studies the strains of *Saccharomyces cerevisiae* were grown in the medium of Billen and Lichstein(6) and strains of *Saccharomyces carlbergenesis* in medium of Atkin *et al.*(7). Following seeding with a 24-hour-old culture and solidification of the agar, discs containing 100 μg of drug (except for Actinomycin D, 10 μg) were placed on the surface. The dishes were incubated at 28° for 24-72 hours, depending upon the strain. The diameter of each zone of inhibition was measured in millimeters.

TABLE I. Results of Inhibition Tests Using a Selected Series of Compounds.

Drug	Yeasts	*Staphylococcus aureus*
	Inhibitory index*	
5-Fluorouracil	35/50	40†/34
5-Fluorodeoxyuridine	23/20	25/22
N,N',N''-Triethylene thiophosphoramide	17/20	0/0
Methyl bis (β chloroethyl) amino	28/30	0/0
A-methopterin	45/45	20/5
8-azaguanine	44/10	0/0
Azaserine	19/20	16/16
Actinomycin D	0/0	24/38
Diethylstilbestrol	0/0	20/18
Nitromin	0/0	0/15

* Zone of inhibition in mm of parent/zone of inhibition in mm of mutant.
† Partial zone.

For the *S. aureus* tests the cells were grown for 18-24 hours at 37° in brain-heart infusion broth. *S. aureus* (ATCC #13679) was diluted 1:100 while the "mutants" were diluted 1:10. One ml of cell suspension was mixed with brain-heart infusion agar. Discs identical to those used with the yeast were placed on the hardened agar. After 24 hours' incubation at 37°, zones of inhibition were evaluated as above.

Results. The following compounds, at the concentrations used, did not inhibit any of the yeasts or staphylococci: estrone, urethan, 6-chloropurine, hydrocortisone, 6-mercaptopurine and AB-103 (Benzyl N-bis(ethylenimido)phosphorocarbamate). The results for yeast are grouped together because neither yeast strain nor mutant was more sensitive than any other. One compound, 5-fluorouracil, selectively caused greater inhibition of yeast mutants and staphylococci "mutants" than the parental types.

The remaining yeast results show the same degree of inhibition for mutants and parents except for 8-azaguanine which preferentially inhibited the parental types. The staphylococcal "mutants" were selectively inhibited by nitromin, relatively more inhibited by actinomycin D and less inhibited by A-methopterin than was the parent. No differ-

ences were noted with the remaining compounds.

Discussion. The results presented can not be considered as substantiating evidence endorsing the use of this test as a primary screen for cancer chemotherapy. Thus far, additional results with about 700 compounds including 150 antibiotic beers have been obtained using several yeast species and respiratory mutants obtained from them. In those cases in which occurred preferential inhibition of the mutants and not of the parents, the compound was tested in several *in vivo* experimental animal tumor systems. No compound was an effective tumor inhibitor. The yeast study did not indicate any class of compound as being particularly inhibited.

Additional study of Gause's staphylococci (ATCC #13679-13681) is not contemplated. Gause claims that strain 13680 and 13681 are respiratory "mutants" produced by ultraviolet irradiation of *S. aureus* 13679. While the present study verified his findings that these strains have a Q_{O_2} of approximately 48 and 42% that of the parent culture, the parent strain and the "mutants" were found to differ in several respects. Visibly, 13679 produced typical golden colonies while the substrains produced pink colonies. A typical *S. aureus* enzyme, coagulase, was not present in the substrains. Further differences were found in the growth characteristics of "mutants" and the parent strain. The parent grew overnight with 7.5% sodium chloride in the medium, or in tryptose-glucose broth and produced acid but no gas from mannitol. The substrains grew poorly after one week with 7.5% sodium chloride in medium, after 3 days in tryptose-glucose broth, and produced neither acid nor gas from mannitol after one week. Neter *et al.*(8) found large amounts of Rantz antigen in the supernates of the parent strain in contrast to the mutants. Furthermore, only the supernates of the parent strain inhibited agglutination by *S. aureus* and *L. monocytogenes* anti-sera of erythrocytes modified by either *B. subtilis* or *L. monocytogenes* antigens.

Since mutants obtained by ultraviolet irradiation are usually considered to be altered at a single enzymatic locus, it seems unlikely that a change in a single enzyme should have led, in this instance, to such extensive differences between parent and substrains. Strains 13680 and 13681 probably represent contaminants of the original culture of *S. aureus* 13679.

Summary. Respiratory deficient mutants of yeast, obtained by incorporating acriflavine into the growth medium, as well as *S. aureus* "mutants" obtained from ATCC were used for testing cancer chemotherapeutic agents. Although there is a feasible theory that supports the use of a respiratory mutant with a change in the mode of utilization of glucose, the procedure described appears to be of limited value. Examination of the *S. aureus* mutants (ATCC #13680-13681) from Gause lead to doubt of their authenticity.

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